Effects of selected pesticides and adjuvants on germination and vegetative growth of *Phomopsis amaranthicola*, a biocontrol agent for *Amaranthus* spp.

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Summary

Phomopsis amaranthicola, a bioherbicide agent for *Amaranthus* spp., was tested *in vitro* for its compatibility with commercial formulations of 16 adjuvants, 24 herbicides, nine fungicides and four insecticides at 2X, 1X (highest labelled product dose rate), 0.75X, 0.5X and 0.25X concentrations. These chemicals were tested for their effects on spore germination. Selected herbicides and fungicides at 1X were also tested for their influence on colony growth and sporulation. All tested compounds were finally categorized in compatibility classes regarding germination, mycelial growth and sporulation such as low, medium and high compatibility. High compatibility in terms of germination and fungal colony growth was only observed with the herbicide bentazone. Many of the adjuvants tested were highly compatible in the germination studies. Most fungicides and insecticides had medium and low compatibility with *P. amaranthicola*. Effects of pesticides on spore germination did not fully correspond with their effects on colony growth. Thus, it is possible to integrate the use of *P. amaranthicola* with some herbicides, fungicides, insecticides and adjuvants but not others. A case-by-case selection of application methods, such as tank-mix or sequential application, along with proper timing of applications of the fungus and the chemical agents will be necessary.

Keywords: adjuvant, *Amaranthus* spp., bioherbicide, biological control, compatibility, herbicide, fungicide, insecticide, *Phomopsis amaranthicola*.

Introduction

Amaranthus spp. and especially *A. retroflexus* L., *A. hybridus* L., *A. viridus* L. and A *spinosus* L. are considered major weeds not only in many field and vegetable crops around the world but also in ruderal sites, roadsides and fallow fields (Bürki *et al.*, 1997; Holm *et al.*, 1977, 1997). The problems with *Amaranthus* spp. have been accentuated because of the development of weed biotypes resistant to s-triazine herbicides (Bandeen *et al.*, 1982), multiple resistance to triazine and acetolactate synthase (ALS) herbicides (Foes *et al.*, 1998) and cross-resistance to dinitroaniline herbicides (Gossett *et al.*, 1992). *Amaranthus* spp. also have the ability to invade their habitats quickly because of a large number of very small (1–1.5 mm diameter), long-lived seeds that are dispersed by wind and water (Stevens, 1932). As C4 plants, they succeed with superior growth rates relative to competing C3 plants such as crops and other weeds (Pearcy & Ehleringer, 1984). The competitive ability of *Amaranthus* plants has been well documented in a number of cropping systems [e.g. maize (*Zea mays* L.) (Holm *et al.*, 1997), cotton (*Gossypium hirsutum* L.) (Buchanan *et al.*, 1980) and potatoes (*Solanum tuberosum* L.) (VanGessel & Renner, 1990)].

Conventional control of *Amaranthus* spp. has depended on application of chemical herbicides and cultural means, such as increased tillage. Currently, there are a number of herbicides that are effective for the control of *Amaranthus* spp., although the majority of the herbicides are registered for use in specific crops such as cereals, turf and major vegetables, very few are registered for use in minor crops (Stall *et al.*, 1998). In addition, in many countries, pesticide policies have

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called for significant reductions in the use of chemical pesticides together with promotion of biodiversity in agro-ecosystems. The interest in alternative weed control methods is therefore increasing. For instance, Ammon (1994) demonstrated that cover crops in maize can reduce *A. retroflexus* populations by 90%, however, the surviving plants produced more seeds per plant, increasing the possibility of dense stands of the weed in the following year (Bürki *et al.*, 1997).

Phomopsis amaranthicola Rosskopf, Charudattan, Shabana, et Benny sp. nov. is a coelomycetous, pycnidial fungus that causes a stem- and leaf-blight on *Amaranthus* spp. It is an effective broad-spectrum bioherbicide agent for *Amaranthus* spp. and produces three types of conidia, α, β and C. Leaf symptoms are apparent 5–7 days after inoculation and the disease leads to premature leaf abscission, stem constriction and toppling of plants (Wyss & Charudattan, 1999; Rosskopf *et al.*, 2000b). Its efficacy as a biocontrol agent was confirmed under controlled environmental conditions in a greenhouse and in the field (Rosskopf, 1997). Conidial suspensions were more effective than mycelial suspensions. All species tested such as *A. lividus* L., *A. viridus*, *A. hybridus, A. retroflexus* and *A. spinosus* were controlled at 50–100% in one or more years during a 3-year test period (Rosskopf, 1997; Rosskopf *et al.*, 2000a).

The effect of natural and synthetic chemical pesticides on biocontrol agents is an important element in the development of integrated pest management strategies (Charudattan, 1993). A major concern is whether a biocontrol agent can be integrated within the normal pesticide spraying schedule used for crop protection. Compatibility of herbicides with fungal biological control agents has been examined to some extent (Khalil *et al.*, 1985; Grant *et al.*, 1990a,b; Poprawski & Majchrowicz, 1995; Rayachhetry & Elliott, 1997; Wyss & Müller-Schärer, 2001). In addition to suppressing the activities of biocontrol agents, pesticides can also have synergistic effects (i.e. by reducing host defence responses) (Hoagland, 1996).

It is often necessary to use various types of adjuvants to improve the performance of a biocontrol agent. For instance, adjuvants can improve spore adhesion, spread the inoculum evenly on leaf surfaces, or protect infection structures from adverse environmental influences such as UV-irradiation (Greaves & MacQueen, 1990; Womack & Burge, 1993). Nonetheless, certain adjuvants can be toxic to certain fungi. Therefore, adjuvants used with a fungus must be tested for toxicity to that particular strain both singly and in combination with any other additive which is to be included in the final formulation. Moreover, adjuvants might induce phytotoxicity on the weed host or crop plant, both by altering the morphology of the epicuticular wax and by damaging leaf tissue (Falk *et al.*, 1994).

How pesticides and adjuvants may affect the performance of *P. amaranthicola* is unknown. Hence, the primary purpose of this investigation was to determine whether herbicides, fungicides, insecticides and selected adjuvants used at different doses affected conidial germination of *P. amaranthicola*, and to develop the types of initial estimates of compatibility that *in vitro* studies will provide. Selected pesticides were also tested at the highest rates for their effects on vegetative growth and sporulation of *P. amaranthicola*.

Materials and methods

Fungal culture

A single-spore isolate of *P. amaranthicola* (ATCC 74226) used in this study was isolated in 1992 from a naturally infected *A. hybridus* plant found in Gainesville, FL, USA (Rosskopf *et al.*, 2000b). Stock cultures of *P. amaranthicola* were maintained in sterilized soil in glass tubes that had been inoculated with a spore suspension and stored at 10°C in the dark. For inoculum preparation, a few milligrams of soil was sprinkled on V8 plates. After 12 days, newly developed mycelium was transferred to fresh V8 plates and grown for 2–3 weeks at 24 $\pm$ 1°C with a 12-h photoperiod under fluorescent light with a photon flux density of 20 μmol m^{-2} s^{-1}. Agar plugs and conidial spore suspensions prepared from the V8 plates were used as inoculum.

Pesticides and adjuvants used

Twenty-four herbicides, nine fungicides and four insecticides (Table 1) were tested at five concentrations: 0.25X, 0.5X, 0.75X, 1X (= highest labelled product rate from among all uses) and 2X, as well as 16 adjuvants at the labelled product rates (Table 2) for their effects on germination of α-conidia of *P. amaranthicola*. For the 1X rates, the highest product rates [HR] labelled for any crop in which *Amaranthus* spp. cause problems were chosen. In addition, selected herbicides and fungicides were tested at 1X for their effect on vegetative growth and sporulation of *P. amaranthicola* on V8 agar in Petri dishes. All preparations were calculated based on an application volume of 468 L ha^{-1}.

The choice of pesticides and adjuvants selected was based on a pre-selection and on their regular use in crop production systems for vegetables, sugarcane, pine nurseries and turf grasses in Florida where *Amaranthus* spp. are problem weeds. We have considered insecticides to be less of a problem than herbicides and fungicides for the purpose of integration of the fungus in a

Table 1 Fungicides, herbicides and insecticides used, their formulation, and dose rate for compatibility studies with *Phomopsis amaranthicola*

Common name	Trade name	Type or modes of action	Supplier*	a.i. (g kg^{-1})**	Formulation	Highest labelled product rate (X) (kg ha^{-1})***
Fungicides						
Benomyl	Benlate SP	Systemic, β-tubulin assembly in mitosis	DuPont, Wilmington, DE	505	WP	2.24
Chlorothalonil	Bravo 500	Protectant, multisite activity	Zeneca, Wilmington, DE	500 g L^{-1}	LC	5 L ha^{-1}
Copper hydroxide	Kocide DF	Protectant, enzyme function disruptor	Griffin LLC, Valdosta, GA	614	DF	4.5
Fosetyl-Al	Aliette WDG	Systemic, stimulates plant defences	Bayer CropScience, Kansas City, MO	800	WDG	5.6
Iprodione	Rovral 50	Protectant, NADH cytochrome *c* reductase in lipid peroxidation	Bayer CropScience, Kansas City, MO	500	WP	2.2
Mancozeb	Dithane M-45	Protectant, multisite activity	Dow AgroSciences, LLC, Indianapolis, IN	800	WP	3.4
Maneb	Manex	Protectant, multisite activity	Griffin LLC, Valdosta, GA	480 g L^{-1}	FL	5.6 L ha^{-1}
Metalaxyl/Mancozeb	Ridomil MZ 58	Systemic, inhibition of RNA polymerase I	Syngenta Crop Protection Greensboro, NC	480/100	WP	2.2
Vinclozolin	Curalan DF	Protectant, NADH cytochrome *c* reductase in lipid peroxidation	BASF Corporation, Mount Olive, NJ	500	WDG	3
Herbicides						
Ametryn	Evik 80W	Photosynthesis inhibitor	Syngenta Crop Protection Greensboro, NC	760	WP	1.7
Atrazine	Atrazine 80W	Photosynthesis inhibitor	Syngenta Crop Protection Greensboro, NC	760	WP	0.6
Bensulide	Prefar 4-E	Inhibition of lipid synthesis – not ACCase inhibition	Gowan, Yuma, AZ	480 g L^{-1}	EL	14 L ha^{-1}
Bentazone	Basagran	Photosynthesis inhibitor	BASF Corporation, Mount Olive, NJ	480 g L^{-1}	LC	2.3 L ha^{-1}
Clethodim	Select 2 EC	Lipid-biosynthesis (ACCase) inhibitor	Valent USA Corporation, Walnut Creek, CA	240 g L^{-1}	EC	1.2 L ha^{-1}
Chlorthal-dimethyl	Dacthal	Microtubule assembly inhibition	AMVAC Chemical Corporation, Los Angeles, CA	719 g L^{-1}	WP	23.4 L ha^{-1}
Diuron	Karmex DF	Photosynthesis inhibitor	Griffin LLC, Valdosta, GA	800	DF	4.5
EPTC	Eptam 7-E	Inhibition of lipid synthesis – not ACCase inhibition	Syngenta Crop Protection Greensboro, NC	840 g L^{-1}	EL	3.5 L ha^{-1}
Glyphosate	Roundup Custom	Amino acid (EPSPS) inhibitor	Monsanto Company, St. Louis, MO	648 g L^{-1}	LC	0.43 L ha^{-1}
Imazapyr	Arsenal	Branched-chain amino-acid	BASF Corporation, Mount Olive, NJ	240 g L^{-1}	AS	1.0 L ha^{-1}
Imazethapyr	Pursuit DG	ALS/AHAS inhibitor	BASF Corporation, Mount Olive, NJ	700	DG	0.15
Linuron	Lorox DF	Photosynthesis inhibitor	Griffin LLC, Valdosta, GA	500	DF	3.4
Metolachlor	Dual 8E	Inhibition of cell division	Syngenta Crop Protection Greensboro, NC	959 g L^{-1}	EC	2.2 L ha^{-1}
Metribuzin	Sencor 75 Turf	Photosynthesis inhibitor	Bayer CropScience, Kansas City, MO	750	DF	1.1
Napropamide	Devrinol 50-WP	Inhibition of cell division	United Phosphorus Inc., Trenton, NJ	500	WP	2.2
Naptalam	Alanap-L	Growth regulator	Crompton Uniroyal Chemical, Middlebury, CT	240 g L^{-1}	LC	18.7 L ha^{-1}
Oxyfluorfen	Goal 2XL	Protoporphyrinogen oxidase inhibitor	Dow AgroSciences, LLC, Indianapolis, IN	240 g L^{-1}	EC	2.3 L ha^{-1}
Paraquat dichloride	Gramoxone Extra	Photosystem I energized cell-membrane destroyer	Syngenta Crop Protection Greensboro, NC	300 g L^{-1}	EC	3.5 L ha^{-1}
Pebulate	Tillam 6-E	Inhibition of lipid synthesis - not ACCase inhibition	Syngenta Crop Protection Greensboro, NC	719 g L^{-1}	EC	9.4 L ha^{-1}
Pendimethalin	Prowl 3.3 EC	Microtubule assembly inhibition	BASF Corporation, Mount Olive, NJ	395 g L^{-1}	EC	8.2 L ha^{-1}
Prodiamine	Barricade 65WG	Microtubule assembly inhibition	Syngenta Crop Protection Greensboro, NC	650	WG	2.6

Table 1 (Continued)

Common name	Trade name	Type or modes of action	Supplier*	a.i. (g kg^{-1})**	Formulation	Highest labelled product rate (X) (kg ha^{-1})***
Sethoxydim	Poast	Lipid-biosynthesis (ACCase) inhibitor	BASF Corporation, Mount Olive, NJ	180 g L^{-1}	EC	2.9 L ha^{-1}
Simazine	Princep Liquid	Photosynthesis inhibitor	Syngenta Crop Protection Greensboro, NC	480 g L^{-1}	LC	9.3 L ha^{-1}
Trifluralin	Treflan HFP	Microtubule assembly inhibition	Dow AgroSciences LLC, Indianapolis, IN	480 g L^{-1}	EC	4.7 L ha^{-1}
Insecticides						
Cyromazine	Trigard	Growth regulator, moulting and pupation inhibitor	Syngenta Crop Protection Greensboro, NC	750	WP	0.2
Dicofol	Kelthane 35	Contact poison, electron-transport inhibitor	Dow AgroSciences LLC, Indianapolis, IN	350	WP	1.8
Dimethoate	Cygon 480 E	Acetyl cholinesterase inhibitor	United Agri Products, Greeley, CO	480 g L^{-1}	EC	1.0 L ha^{-1}
Malathion	Malathion 57 EC	Acetyl cholinesterase inhibitor	United Agri Products, Greeley, CO	600 g L^{-1}	EC	3.5 L ha^{-1}

*All suppliers located in the USA.
**For liquids g L^{-1} was used.
***For liquids L ha^{-1} was used.
AS, aqueous solution; DF, dry flowable; DG, dry granule; EC, emulsifiable concentrate; EL, emulsifiable liquid; FL, flowable; LC, liquid concentrate; WDG, water-dispersible granule; WG, wettable granule; WP, wettable powder. ALS, acetolactate synthase; AHAS, acetohydroxyacid synthase; ACCase, acetyl-CoA carboxylase; EPSP, 5-enolpyruvyl shikimate phosphate.

management programme. *Phomopsis amaranthicola* must be applied when *Amaranthus* spp. are still small and are beginning to compete with the crop. During the same time frame herbicides may be applied, but not insecticides which are generally applied later when the crop is much older. Fungicides applied at the seedling stages of the crops or later in the season may reduce the effectiveness of the fungus by completely preventing or slowing the primary infection, disease development and subsequent disease cycles. Therefore, it is important to study the effects of fungicides.

Germination study

Stock solutions of pesticides were prepared at 10X concentration and test concentrations were prepared by serial dilution. Suspensions of *P. amaranthicola* were mixed at a concentration of 10^6 spores mL^{-1} in deionized water with the appropriate concentration of a pesticide at a ratio of 1:9 to obtain 0.25X, 0.5X, 0.75X, 1X and 2X. The adjuvants were simply mixed with a *P. amaranthicola* suspension of *c.* 10^6 spores mL^{-1}. The pesticide-containing and the adjuvant-containing suspensions were allowed to stand for 20 and 45 min, respectively, before they were used. Spore concentration was determined with a haemocytometer. A 0.5-mL suspension of spores plus the test pesticide was spread evenly over the surface of water agar (1.4%) in 9-cm diameter Petri plates with a rubber spatula or, in the case of adjuvants, sprayed with an aerosol sprayer (SPRA-TOOL® 8112; Crown North American Professional Products, Chicago, IL, USA). Control treatments included spore suspension without pesticide or adjuvant.

The plates were arranged randomly in a single layer and incubated for 24 h at 24 ± 1°C under light at 20 μmol m^{-2} s^{-1} photon flux density. Germination percentage was determined by counting 50 α-conidia on each of the three replicated plates using a light microscope. Conidia were considered germinated when the germ tube was longer than the spore diameter. The pesticide and adjuvant experiments were performed twice.

Culture-growth study and sporulation

In the growth-inhibition tests, the herbicides atrazine, bensulide, bentazone, diuron, EPTC, glyphosate, imazapyr, linuron, metolachlor, naptalam, oxyfluorfen, paraquat, pebulate, pendimethalin, sethoxydim, simazine and trifluralin, as well as the fungicides benomyl, iprodione and maneb were added at HR to molten, sterilized V8 agar. The agar was amended with antibiotics (20 mL L^{-1} each of 2.5 mg mL^{-1} chloramphenicol and 3.7 mg mL^{-1} streptomycin sulphate (Sigma Chemicals, St Louis, MO, USA). One 0.75 cm-diameter

Table 2 Adjuvants and surfactants tested with conidia of *Phomopsis amaranthicola*

Adjuvant	Rate (%)	Chemical composition	Product source*
80 Additive	0.003	Isopropanol and nonylphenol ethoxylate	Brewer International, Vero Beach, FL
Big Wet	0.001	Non-disclosed; blend of anionic and nonionic wetting agent	Brewer International, Vero Beach, FL
Invert Emulsion	**	Paraffinic petroleum oils/light mineral oil (concentrate)	Sun Company, Philadelphia, PA
I'VOD	0.003	D-limonene terpene hydrocarbons and nonylphenol ethoxylate	Brewer International, Vero Beach, FL
Kelgin LV	0.05	Algal polysaccharide composed of sodium polymannuronate	NutraSweet Kelco Company, San Diego, CA
Keltrol HP	0.05	Bacterial polysaccharide composed of D-glucosyl, D-mannosyl, D-glucosyluronic acid, O-acetyl, pyruvic acid acetal	NutraSweet Kelco Company, San Diego, CA
Metamucil	0.5	Psyllium hydrophilic mucilloid, a plant-derived polysaccharide	Procter & Gamble, Cincinnati, OH
Natrosol	0.05	Hydroxyethyl cellulose_1	Aqualon, Wilmington, DE
N-Gel	0.5	Hydroxyethyl cellulose_2	Hercules, Wilmington, DE
Pelgel	0.5	Carboxymethyl cellulose and acacia gum (a complex mixture of polysaccharides)	Lipha Tech., Milwaukee, WI
Poly Control	0.25	Polymer composed of 2-propenamide (1)	Brewer International, Vero Beach, FL
SilEnergy	0.04	Polyalkyleneoxide-modified polydimethylsioloxane	Brewer International, Vero Beach, FL
Silwet L-77	0.25	Polyalkyleneoxide-modified heptamethyltrisiloxane	Loveland Industries, Greeley, CO
Soil Moist	0.5	2-Propenamide copolymer (2)	JRM Chemical, Cleveland, OH
Stocksorb 310K	0.5	Acrylamide potassium acrylate copolymer	Southern Agricultural Insecticides, Inc., Palmetto, FL
Tergitol NP-10	0.25	Nonoxynol	Union Carbide Crop., New York, NY
Triton CS-7	0.25	Alkylaryl polyethoxylate, sodium salt of alkylsulfonated alkylate	Rohm and Haas, Philadelphia, PA
Triton X-100	0.05	Octylphenoxy polyethoxyethanol	Rohm and Haas, Philadelphia, PA
Tween 20	0.3	Polyoxyethylene sorbitan monolaurate	Sigma Chemical, St Louis, MO

*All suppliers located in the USA.
**40% Sunspray 6 agricultural oil (Sun, Marcus Hook, PA), 10% light mineral oil (Fisher Scientific, Atlanta, GA) and 1% Myverol (Eastman Chemical, Kingsport, TN).

agar disc from a 14-day-old culture of *P. amaranthicola* was then placed in the centre of each plate. A control treatment without pesticide was included in each experiment. There were four replicated Petri dishes per pesticide tested. The effect of pesticides and adjuvants on cultural growth was determined from colony diameter measured at 2-day intervals from 2 to 20 days and on the 21st day after inoculation (DAI). Conidial production was assessed 21 DAI by flooding the plates with 10 mL of deionized water. The colony surface was carefully rubbed with a rubber spatula and the conidia were passed through a double layer of cheesecloth. The concentration of conidia was determined with the aid of a haemocytometer and the number of conidia was reported as spores per millilitre. The experiment was conducted twice each in two series (series 1: control, atrazine, benomyl, diuron, glyphosate, imazapyr, maneb, oxyfluorfen, sethoxydim, simazine and trifluralin; series 2: control, bensulide, bentazone, EPTC, iprodione, linuron, metolachlor, naptalam, paraquat, pebulate and pendimethalin).

Statistical analysis

The influence of pesticides on germination of conidia of *P. amaranthicola* was evaluated with the PROBIT procedure of SAS (SAS version 6; SAS Institute, Cary, NC, USA) to relate toxicity of a pesticide (or mortality of conidia) to its product dose rate. The relationship is

$$\text{Probability of mortality} = \Phi\left[\frac{(x - \text{LD}_{50})}{C}\right]$$

where x is the product dose rate of the pesticide, LD_{50} is the product rate where 50% of the conidia are killed and C is a relative concentration parameter respectively. The function Φ is the cumulative distribution function of the standard normal probability distribution. The function $F(x) = \Phi((x - LD_{50})/C)$ is called the PROBIT curve for the pesticide. The PROBIT curve is obtained by using maximum likelihood estimates, LD_{50} and C. This is also the reason why there is no concept of degrees of freedom. Data of repeated experiments could have been pooled. However, for easier presentation and comparison of LD_{50}, the values are given separately for each experiment. Some of the computed LD_{50} values in Table 3 are >2X HR, which seems inappropriate. These values were given because the LD_{50} value of the second experiment was <2X HR. Some of the results are presented graphically (Figs 1–3) where the response to the product rates is shown as mortality of spores. Mortality is calculated as one minus fraction of spores germinated.

The adjuvant data were subjected to analysis of variance (ANOVA, Sokal & Rohlf, 1981), using the General Linear Models procedure of SAS, to test

Table 3 Calculated $LD_{50}(X)$ on conidia germination of *Phomopsis amaranthicola* under the influence of chemicals that are used in crops in which *Amaranthus* species cause problems

	Experiment 1		Experiment 2	
Common name	$LD_{50}(X)$*	SED	$LD_{50}(X)$*	SED
Fungicides				
Benomyl	2.2	0.19	1.9	0.10
Vinclozolin	2.0	0.09	1.5	0.07
Fosetyl-Al	1.1	0.03	0.8	0.02
Chlorothalonil	NG		NG	
Copper hydroxide	NG		NG	
Iprodione	NG		NG	
Mancozeb	NG		NG	
Maneb	NG		NG	
Herbicides				
Atrazine	NLE		NLE	
Bentazone	NLE		NLE	
Imazethapyr	NLE		NLE	
Metribuzin	NLE		NLE	
Napropamide	RG at 2X		RG at 2X	
Prodiamine	RG at 2X		RG at 2X	
Simazine	RG at 2X		RG at 2X	
Ametryn	2.8	0.22	1.9	0.07
Pendimethalin	2.0	0.08	1.9	0.06
Imazapyr	1.9	0.11	1.4	0.06
Chlorthal-dimethyl	1.4	0.05	1.6	0.05
Naptalam	1.0	0.04	0.9	0.04
Sethoxydim	0.8	0.02	0.7	0.02
Trifluralin	0.7	0.01	0.8	0.01
Metolachlor	0.6	0.02	0.5	0.02
Glyphosate	0.6	0.01	0.6	0.02
Clethodim	0.4	0.02	0.4	0.01
Paraquat	0.3	0.02	0.5	0.02
Oxyfluorfen	0.2	0.01	0.2	0.01
Pebulate	0.2	0.01	0.2	0.01
Bensulide	NG		NG	
Diuron	NG		NG	
EPTC	NG		NG	
Linuron	NG		NG	
Insecticides				
Cyromazine	RG at 2X		RG at 2X	
Malathion	0.5	0.01	0.5	0.01
Dimethoate	0.2	0.01	0.4	0.01
Dicofol	NG		NG	

*Concentration range tested from 0X (no pesticide; control) to 2X (X = highest labelled product rate).
NLE = no lethal effect of the chemical at any of the concentrations tested.
RG at 2X = reduced germination only at two times highest labelled rate
NG = no germination at all concentrations tested.
$LD_{50}(X)$, given as fraction of spores germinated at highest labelled product rate, was calculated using the PROBIT model of SAS.

significant differences between the main effects of adjuvant.

Vegetative fungal growth on plates with herbicides and fungicides was analysed by random coefficient regression analysis (Gumpertz & Pantula, 1989). The results were presented as slopes of mean values expressing the diametric colony growth in millimetres per day. Pesticides were tested in two series, but because of differences among experiments, the mean values are given separately for the two experiments of each series.

Data on sporulation was not subjected to statistical analysis due to the high variation. Mean values of eight replicated Petri dishes, 16 in the case of the control without amendment of a pesticide, were calculated. A spore reduction rate was calculated following the formula

$$\text{Spore reduction rate}[\%] = \frac{100(Sp_c - Sp_p)}{Sp_c}$$

where Sp_c represents spores produced on the control and Sp_p spores produced in the presence of pesticides.

In Table 5, compatibility classes for influences of pesticides and adjuvants on germination of conidia, fungal colony growth and sporulation were determined based on the following criteria.

Pesticides	Compatibility classes
Germination	'High' means fully compatible with the pesticide tested or reduced at 2X only, LD_{50} (X) from 2.99 to 2.00; 'medium' means that germination and mycelial growth were reduced, LD_{50} (X) from 1.99 to 1.00; 'low' means no compatibility or strong inhibition of germination and mycelial growth, LD_{50} (X) from 0.99 to 0.00. Averages from the two experiments were used for the assignment to the classes
Fungal colony growth	'High' means that diametric colony was comparable with the control; 'medium' means that growth was restricted; 'low' means that there was no growth at all. Averages from the two experiments were used for the assignment to the classes
Sporulation	'High' means that sporulation was close to the water control: 'medium' means that sporulation was at a low level; 'low' means that there was no sporulation at all
Adjuvants	
Germination	'High' means 90–100% germination; 'medium' means 80–90% germination; 'low' means <80% germination. Averages from the two experiments were used for the assignment to the classes

Results

Germination study

Most of the fungicides (chlorothalonil, copper hydroxide, iprodione, mancozeb and maneb) inhibited spore

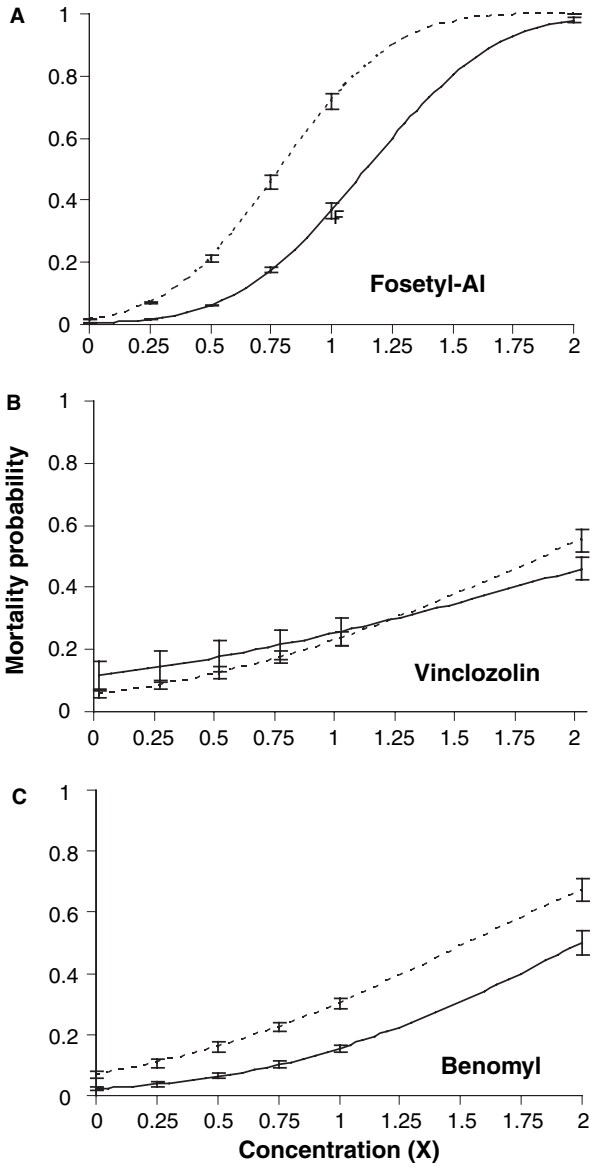

Fig. 1 Mortality probability of spores of *Phomopsis amaranthicola* influenced by the fungicides fosetyl-Al, vinclozolin, and benomyl from 0X (pesticide-free control) to 2X (1X = highest labelled product rate) in Experiment 1 (solid line) and Experiment 2 (dotted line) (fitted PROBIT curve). A: Fosetyl-Al $LD_{50Exp1} = 1.1$, $LD_{50Exp2} = 0.8$; B: Vinclozolin $LD_{50Exp1} = 2.0$, $LD_{50Exp2} = 1.5$; C: Benomyl $LD_{50Exp1} = 2.2$, $LD_{50Exp2} = 1.9$. Chlorothalonil, copper hydroxide, iprodione, mancozeb, and maneb also were tested and were toxic at all concentrations (data not shown). Vertical bars represent ± SE.

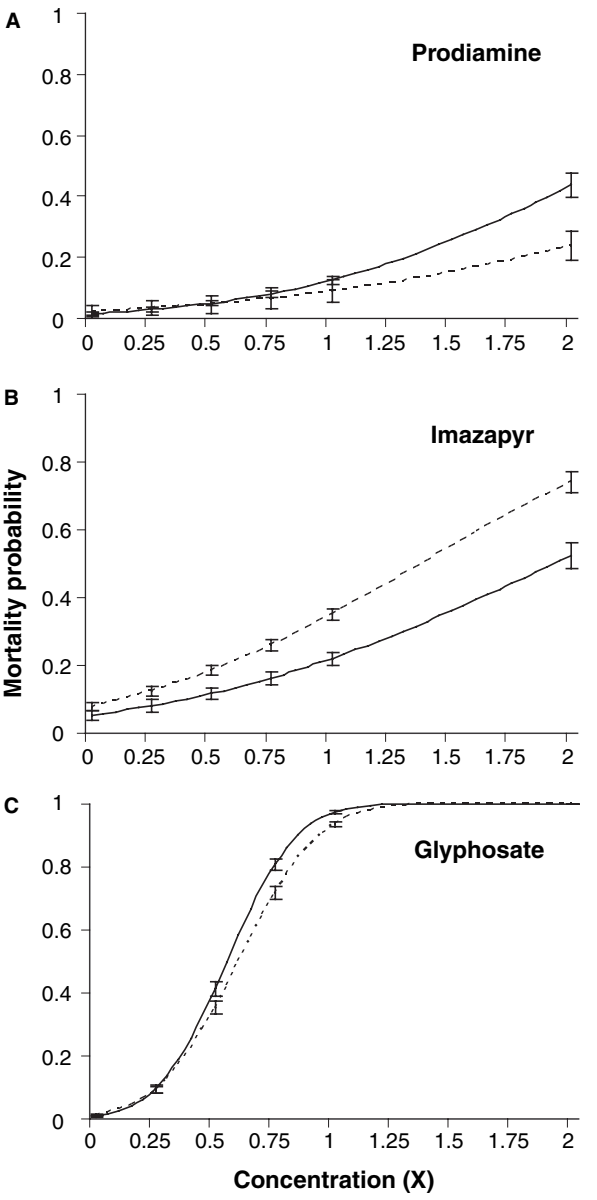

Fig. 2 Mortality probability of spores of *Phomopsis amaranthicola* influenced by the herbicides prodiamine, imazapyr and glyphosate from 0X (no pesticide; control) to 2X (X = highest labelled product rate) in Experiment 1 (solid line) and Experiment 2 (dotted line) (fitted PROBIT curve). Prodiamine represents an example for the compatibility class 'high' (LD_{50} (X) from 2.99 to 2.00); imazapyr represents the class 'medium' (LD_{50} (X) from 1.99 to 1.00), and glyphosate represents the class 'low' (LD_{50} (X) from 0.99 to 0.00) (see also Tables 3 and 5). Vertical bars represent ± SE.

germination at all concentrations (Table 3). In contrast, the LD_{50} values for fosetyl-Al, benomyl and vinclozolin were in excess of 1X HR, except for fosetyl-Al in Experiment 2 (Table 3 and Fig. 1). Benomyl at ≥0.5X HR caused thicker germ tubes compared with the control (data not shown).

The herbicides tested influenced germination of *P. amaranthicola* to varying degrees. Bensulide, diuron, EPTC and linuron inhibited germination at all concentrations (Table 3). In contrast, atrazine, bentazone, imazethapyr and metribuzin at the concentrations tested did not affect germination. Whereas napropamide, prodiamine and simazine affected germination but only at 2X HR (Table 3). Germination was almost unaffected at 1X and weakly affected at 2X HR with ametryn,

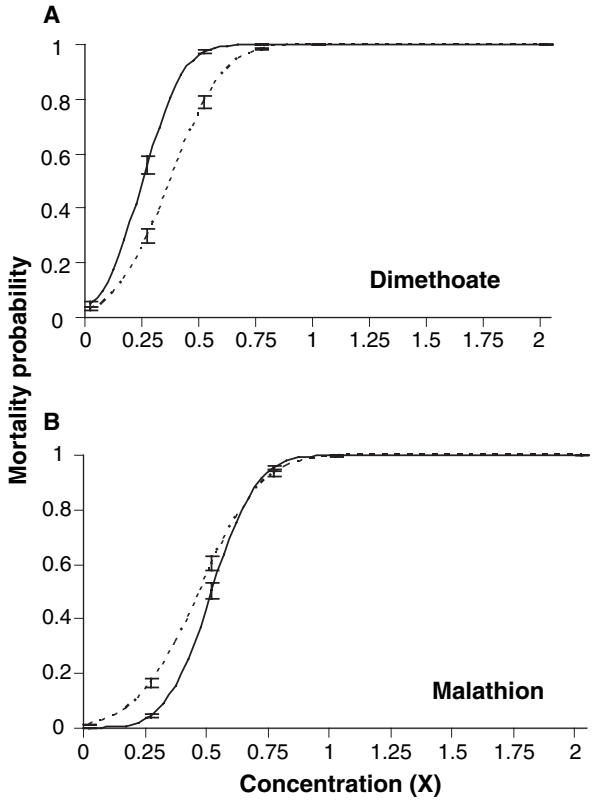

Fig. 3 Mortality probability of spores of *Phomopsis amaranthicola* influenced by the insecticides dimethoate and malathion from 0X (pesticide-free control) to 2X (1X = highest labelled product rate) in Experiment 1 (solid line) and Experiment 2 (dotted line) (fitted PROBIT curve). A: Dimethoate $LD_{50Exp1} = 0.2$, $LD_{50Exp2} = 0.4$; Malathion $LD_{50Exp1} = 0.5$, $LD_{50Exp2} = 0.5$. B: Cyromazine showed reduced germination at 2X only and dicofol was toxic at all concentrations tested (data not shown). Vertical bars represent ± SE.

chlorthal-dimethyl, imazapyr, naptalam and pendimethalin (Table 3 and Fig. 2). Glyphosate, metolachlor, sethoxydim and trifluralin reduced spore germination by 50% at concentrations of 0.5X and 0.75X HR respectively (Table 3 and Fig. 2). An LD_{50} value below 0.5X HR was observed with clethodim, paraquat, oxyfluorfen and pebulate (Table 3 and Fig. 3). Some of the herbicides also influenced germ-tube development. Stunted and shorter germ tubes in comparison with the control were observed with ametryn, EPTC, fosetyl-Al, napropamide, naptalam and trifluralin at ≥1X HR. Germ tubes were even shorter at 0.25X HR with metolachlor compared with the control. In the case of paraquat, germ tubes were generally shorter, with thinner tips compared with a normally grown germ tube (data not shown).

Spore germination was highly affected by the insecticides dimethoate and malathion (Fig. 3) and conidia were killed at all concentrations by dicofol (Table 3). Cyromazine reduced conidia germination only at 2X HR (Table 3).

In both experiments, germination of conidia under the influence of adjuvants differed significantly among the treatments (ANOVA $P < 0.05$). In both experiments, 0.25% polyalkyleneoxide-modified heptamethyltrisiloxane inhibited germination completely and 0.25% nonoxynol and 0.25% alkylaryl polyethoxylate/sodium salt of alkylsulfonated alkylate caused a 75% reduction of germination in comparison with the control (Fig. 4). The increased germination when compared with the control in 0.3% polyoxyethylene sorbitan monolaurate, 0.5% acrylamide potassium acrylate copolymer, the paraffinic petroleum oils/light mineral oil, 0.5% 2-propenamide copolymer (2) and 0.05% algal polysaccharide, found in the first experiment, was not repeated in Experiment 2.

Culture-growth study and sporulation

Experiments were analysed separately because of the significant experiment-by-pesticide interaction (data not

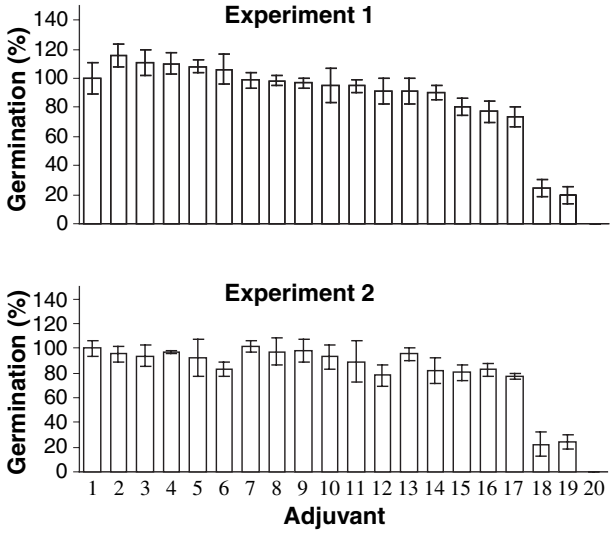

Fig. 4 Influence of adjuvants on germination of conidia of *Phomopsis amaranthicola*. *X*-axis: 1, control treatment; 2, polyoxyethylene sorbitan monolaurate; 3, acrylamide potassium acrylate copolymer; 4, paraffinic petroleum oils/light mineral oil; 5, 2-Propenamide copolymer (2); 6, algal polysaccharide composed of sodium polymannuronate; 7, hydroxyethyl cellulose_1; 8, D-limonene terpene hydrocarbons and nonylphenol ethoxylate; 9, isopropanol and nonylphenol ethoxylate; 10, blend of anionic and nonionic wetting agent; 11, polymer composed of 2-propenamide (1); 12, polyalkyleneoxide modified polydimethyl-sioloxane; 13, octylphenoxy polyethoxyethanol; 14, psyllium hydrophilic mucilloid; 15, hydroxyethyl cellulose_2; 16, bacterial polysaccharide; 17, carboxymethyl cellulose and acacia gum; 18, nonoxynol; 19, alkylaryl polyethoxylate/sodium salt of alkylsulfonated alkylate; 20, polyalkyleneoxide-modified heptamethyltrisiloxane. Data are the means of three Petri dishes per treatment. The experiment was repeated once. Fifty spores each were counted for germination. Vertical bars represent ± SE of the mean. Germination rate of the control was set at 100%.

shown). In series 1, Experiment 1, *P. amaranthicola* was able to grow on V8 amended with imazapyr, sethoxydim, atrazine and oxyfluorfen at HR but at a significantly reduced rate compared with the control. In Experiment 2, there was no significant growth inhibition for imazapyr and sethoxydim (ANOVA $P < 0.05$). In both experiments of series 1, a consistent large reduction of colony growth was observed with glyphosate, resulting in an atypical, adpressed, yellowish culture. The fungus did not grow on V8 agar with the fungicides benomyl and maneb, and the herbicides diuron, simazine and trifluralin (Fig. 5). Although there was growth on V8 amended with atrazine, imazapyr, oxyfluorfen and sethoxydim, the mycelial growth features were changed by the chemicals. The culture of *P. amaranthicola* appeared white and fluffy with atrazine, oxyfluorfen and sethoxydim, and grey-yellowish and adpressed with imazapyr. Moreover, there was no spore production on agar containing the latter (Table 4).

In both Petri-plate studies of series 2, there was a significantly higher diametric colony growth on V8 amended with bentazone compared with the control treatment (ANOVA $P < 0.05$). The bipyridylium herbicide paraquat affected spore germination even at 0.25X HR, resulting in a very low LD_{50} value. Colony growth, however, was not completely restricted but merely inhibited. Mycelial development was sparse and the mycelium took up the green colour of the paraquat solution. Growth also was disturbed by naptalam.

The fungus did not grow on plates containing the fungicide iprodione or the herbicides bensulide, linuron, metolachlor, pebulate and pendimethalin (Fig. 5). In terms of mycelial growth, bentazone was an exception in that it supported normal *P. amaranthicola* growth, with white mycelium growing in a concentric pattern, on the control plates. However, spore production on plates containing bentazone was not as high as on control plates (Table 4). All other chemicals that inhibited growth of *P. amaranthicola*, such as glyphosate, paraquat and naptalam, also suppressed conidiation (Fig. 5 and Table 4).

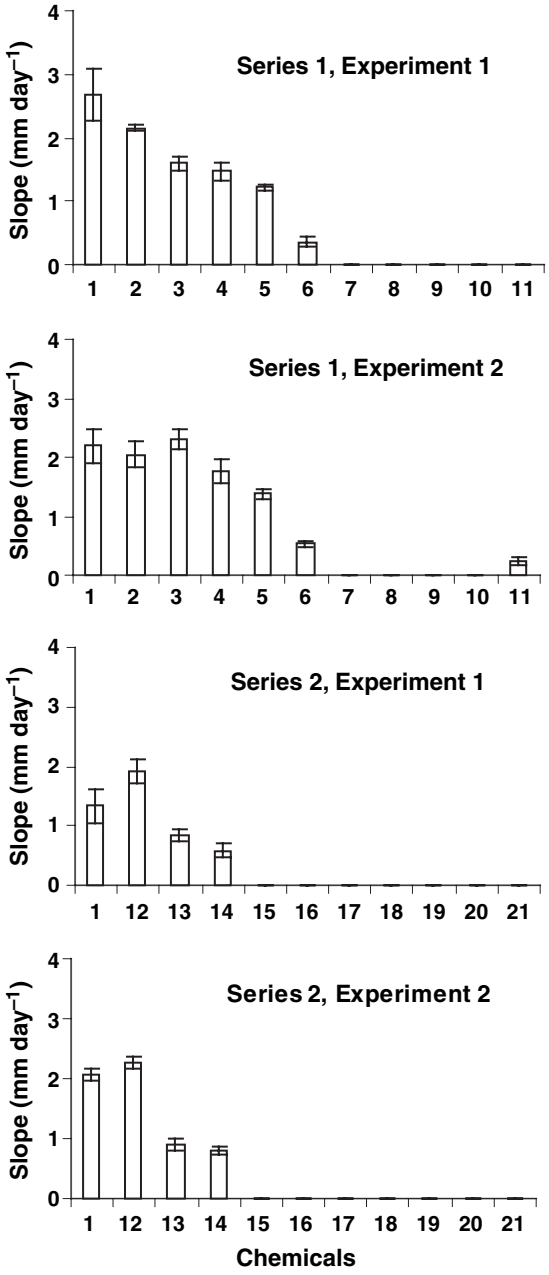

Fig. 5 Diametric colony growth of *Phomopsis amaranthicola* on V8 expressed in millimetre per day representing the slope of mean values amended with pesticides at highest labelled product rate (1, control; 2, imazapyr; 3, sethoxydim; 4, atrazine; 5, oxyfluorfen; 6, glyphosate; 7, benomyl; 8, maneb; 9, diuron; 10, simazine; 11, trifluralin; 12, bentazone; 13, paraquat; 14, naptalam; 15, EPTC; 16, iprodione; 17, bensulide; 18, linuron; 19, metolachlor; 20, pebulate; 21, pendimethalin). Data are the means of four replicate Petri dishes per treatment. The experiment was repeated once. Vertical bars represent ± standard errors of the mean.

Compatibility of pesticides and adjuvants with P. amaranthicola

Pesticides and adjuvants tested for effect on germination of conidia, fungal colony growth and sporulation were categorized in compatibility classes such as high, medium and low compatibility (Table 5).

Generally, compatibility was low with the insecticides tested except cyromazine. In addition, most of the fungicides were placed in the compatibility class 'low'. Benomyl showed high compatibility in the germination study, however, fungal colony growth was completely inhibited. High and medium compatibilities were observed in the germination and culture growth study with the herbicides bentazone and imazapyr respectively. Depending on the developmental stage of the

Table 4 Sporulation of *Phomopsis amaranthicola* on V8 plates amended with pesticides at the highest labelled product rate

Pesticide common name	Sporulation* (spores mL^{-1})	Spore reduction rate (%)
Control	688 542	–
Sethoxydim	404 167	41.3
Oxyfluorfen	189 583	72.5
Atrazine	164 583	76.1
Bentazone	130 476	81.1

*Values given are not based on a statistical analysis due to high variation. They are mean from eight replicated Petri dishes (16 in the case of the control without amendment of a pesticide).
There was no sporulation with the following pesticides: benomyl, bensulide, diuron, EPTC, glyphosate, imazapyr, iprodione, linuron, metolachlor, naptalam, paraquat, pebulate, pendimethalin, simazine and trifluralin.

fungus, some herbicides were highly compatible during germination, others during fungal colony growth.

Most of the adjuvants showed high compatibility or at least medium compatibility which still represents 80–90% germination of conidia. Only alkylaryl polyethoxylate, carboxymethyl cellulose and acacia gum, nonoxynol and polyalkyleneoxide-modified heptamethyltrisiloxane was strongly reduced or completely inhibited germination.

Discussion

In order for a bioherbicide to be used effectively in weed-control programmes, it should be possible to integrate the biological agent with chemical pesticides used in crop production. Any bioherbicide, even if used alone, will have to be resistant to, or tolerant of, herbicides and other pesticides used in the crop production strategy (Greaves & MacQueen, 1990). Hence it is important to define the compatibility of *P. amaranthicola* with pesticides.

Spore germination and culture growth were completely suppressed with the protective fungicides except vinclozolin that allowed 100% growth at 0.25X HR. Spore germination was partially inhibited by fosetyl-Al at concentrations of 0.75X HR or above. The systemic benomyl affected germination at all concentrations but some spores still germinated while culture growth was completely suppressed at HR. In contrast, benomyl at a comparable HR produced only a 66.1% reduction in the growth on potato dextrose agar (PDA) of *Chondrostereum purpureum* Fr./Pouzar, a bioherbicide agent for control of hardwood shrubs and trees (Prasad, 1994). In the study by Grant and colleagues (1990a), germination of conidia of *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *malvae*, a bioherbicide agent for *Malva pusilla* With. (round-leaf mallow), was also affected by most of the fungicides tested. Grant and colleagues (1990a) found that at concentrations of 0.01 and 0.1 times the recommended rate, benomyl, iprodione and mancozeb reduced conidial germination. Spore germination was totally inhibited by chlorothalonil.

Naptalam, the only representative from the group of growth regulators, was highly inhibitory to *P. amaranthicola* even at a concentration of 0.5X HR. Moreover, naptalam at 1X HR reduced the colony growth, changed the colour and growth characteristics of the culture and prevented spore production. The amino acid inhibitor glyphosate behaved similarly to naptalam except that spore germination was completely suppressed at 1X and 2X HR and colony growth highly reduced. In compatibility tests with *C. purpureum*, glyphosate added at 0.1% to PDA (comparable to 0.5X in our study) completely inhibited colony growth (Prasad, 1994). Germination of aeciospores of the rust fungus *Puccinia lagenophorae* Cooke, a biocontrol agent for *Senecio vulgaris* L., was suppressed completely by glyphosate even at rates of 0.0125 and 0.025 times the recommended rate, which are sublethal to plants (Wyss & Müller-Schärer, 2001).

Imazapyr and imazethapyr, both imidazolinone herbicides, had no effect on spore germination or had an effect only above HR. The development of *P. amaranthicola* on agar exposed to imazapyr at HR was no different from the control in one of the experiments and there was no spore production. Imazethapyr at 0.01, 0.1, 0.5, 1 and 2 times the recommended rate affected spore germination of *C. gloeosporioides* f. sp. *malvae*, although germination remained above 50% of the control at all concentrations (Grant *et al.*, 1990b).

Clethodim and sethoxydim belong to the cyclohexanedione herbicides. Both herbicides were inhibitory even at 0.5X HR and conidial germination was suppressed completely at HR and above. Colony growth of *P. amaranthicola* was inhibited by sethoxydim in Experiment 1 only, whereas spore production was similar to that of the control. On the contrary, exposure of *C. gloeosporioides* f. sp. *malvae* to sethoxydim had a more adverse effect on spore germination than on growth. At 0.5 times the recommended field rate, spore germination dropped to 0%, whereas mycelial development was inhibited only at the recommended rate and not at 0.01, 0.1 and 0.5 times the field rate (Grant *et al.*, 1990b).

The exposure to the triazine herbicides ametryn, atrazine, metribuzin and simazine resulted either in no or only a slight reduction in germination of *P. amaranthicola* at 2X HR. Colony growth and spore production, however, were inhibited completely by simazine and significantly by atrazine. Metribuzin

Table 5 Compatibility classes (high, medium and low) for all tested compounds with regard to germination, mycelial growth and sporulation of *Phomopsis amaranthicola*

	Compatibility classes		
	High	Medium	Low
Germination*			
Fungicides	Benomyl	Vinclozolin	Chlorothalonil, copper hydroxide, fosetyl-Al, iprodione, mancozeb, maneb
Herbicides	Ametryn, atrazine, bentazone, imazethapyr, metribuzin, napropamide, pendimethalin prodiamine, simazine	Chlorthal-dimethyl, imazapyr, naptalam	Bensulide, clethodim, diuron, EPTC, glyphosate, linuron, metolachlor, oxyfluorfen, paraquat, pebulate, sethoxydim, trifluralin
Insecticides	Cyromazine		Dicofol, dimethoate, malathion
Adjuvants	Acrylamide potassium acrylate, blend of anionic and nonionic wetting agent, paraffinic petroleum oils/light mineral oil, hydroxyethyl cellulose_1, isopropanol and nonylphenol ethoxylate, D-limonene terpene hydrocarbons and nonylphenol ethoxylate, octylphenoxy polyethoxyethanol, polymer composed of 2-propenamide (1), polyoxyethylene sorbitan monolaurate, 2-propenamide copolymer (2), algal polysaccharide	Hydroxyethyl cellulose_2, polyalkyleneoxide-modified polydimethylsioloxane, psyllium hydrophilic mucilloid, bacterial polysaccharide	Alkylaryl polyethoxylate, carboxymethyl cellulose and acacia gum, nonoxynol, polyalkyleneoxide-modified heptamethyltrisiloxane
Mycelial growth†			
Fungicides			Benomyl, maneb, iprodione
Herbicides	Bentazone, imazapyr	Atrazine, glyphosate, naptalam, oxyfluorfen, paraquat, sethoxydim	Bensulide, diuron, EPTC, linuron, metolachlor, pebulate, pendimethalin, simazine, trifluralin
Insecticides	n.a.	n.a.	n.a.
Sporulation‡			
Fungicides		Atrazine, bentazone, oxyfluorfen	Benomyl, iprodione, maneb,
Herbicides	Sethoxydim		Bensulide, diuron, EPTC, glyphosate, imazethapyr, linuron, metolachlor, naptalam, paraquat, pebulate, pendimethalin, simazine, trifluralin
Insecticides	n.a.	n.a.	n.a.

*The compatibility classes for germination influenced by fungicides, herbicides and insecticides are based on the results from Table 3; mean values were calculated from the two experiments and the corresponding pesticides were placed in the appropriate compatibility class.' High' means full compatibility with the pesticide tested or reduced at 2X only, LD_{50} (X) from 2.99 to 2.00. 'Medium' means that germination and mycelial was growth reduced, LD_{50} (X) from 1.99 to 1.00. 'Low' means that there was no compatibility or strong inhibition of germination and mycelial growth, LD_{50} (X) from 0.99 to 0.00.

The compatibility classes for germination influenced by adjuvants are based on Fig. 4. 'High' means 90–100% germination; 'medium' means 80–90% germination; 'low' means $<80\%$ germination.

†This class was based on the ability of diametric colony growth affected by the pesticides (see Fig. 5). Mean values were calculated from the two experiments. 'High' means that diametric colony was comparable to the control; 'medium' means that there was growth but restricted; 'low' means that there was no growth at all.

‡This class was based on the ability to sporulate (see Table 4). Pesticides which did not allow sporulation were placed into 'low'; pesticides which allowed sporulation were placed into 'medium'; and pesticides with sporulation close to the water control were placed into 'high'.

n.a., Not applicable, this type of compound was not tested.

also did not affect spore germination of *C. gloeosporioides* f. sp. *malvae* at concentrations up to 2X HR but caused the mycelial colour to change (Grant *et al.*, 1990b). The substituted ureas, diuron and linuron, also belonging to the class of photosynthesis inhibitors, were highly toxic. There was no colony

growth and essentially no spore production. In the case of *C. gloeosporioides* f. sp. *malvae*, spore germination was decreased with increasing concentration of linuron. Spore production was still possible and cultural growth was normal in comparison with the control treatment (Grant *et al.*, 1990b). Aeciospores of the rust *P. lagenophorae* treated with linuron at 0.25 and 1 times the recommended field rate failed to germinate (Wyss & Müller-Schärer, 2001).

Bentazone, a benzothiadiazole herbicide with rapid plant death effects, showed a stimulating effect on mycelial growth when compared with the control and sporulation was also observed. Spore germination tests on *C. gloeosporioides* f. sp. *malvae* at similar concentrations of bentazone revealed the same effect (Grant *et al.*, 1990b).

This investigation with a selected group of chemicals revealed that herbicides from the group of ALS/AHAS (acetohydroxyacid synthase) inhibitors, rapidly acting photosynthesis inhibitors, as well as pendimethalin, prodiamine, trifluralin and chlorthal-dimethyl affected spore germination more than herbicides belonging to the growth regulators, the 5-enolpyruvyl shikimate phosphate inhibitor, acetyl-CoA carboxylase inhibitors, cell-membrane destroyer, as well as metolachlor, EPTC and pebulate. The classical photosynthesis inhibitors and cell division inhibitors behaved indifferently.

The insecticides tested were toxic even at reduced rates; dicofol was lethal at all concentrations. Only cyromazine affected spore germination at 2X HR. In general, it is assumed that insecticides do not have significant fungicidal effects. This might also be the reason why there are not many studies published in this area (with the exception of Khalil *et al.*, 1985). The high toxicity found with dicofol, dimethoate and malathion could therefore be related more to the chemical of formulations and to a lesser extent to the active ingredient (Smith & Hallett, 2003).

The results reported herein suggest that atrazine, bentazone, imazethapyr and metribuzin could be used selectively in an integrated control programme where *P. amaranthicola* is used. Our data also suggest a high potential for incompatibility between this fungus and the herbicides ametryn, chlorthal-dimethyl, imazapyr, napropamide, naptalam, pendimethalin, prodiamine and simazine at HR as well as with the fungicides benomyl, fosetyl-Al and vinclozolin (at any rate). With chemicals that were highly toxic to *P. amaranthicola* (e.g. clethodim, glyphosate, metolachlor, oxyfluorfen, paraquat, pebulate, sethoxydim, trifluralin, dimethoate and malathion) or where the rates cannot be reduced, split application may be an alternative. In cases where pesticides caused complete spore kill, such as with the herbicides bensulide, diuron, EPTC and linuron, the fungicides chlorothalonil, copper hydroxide, iprodione, mancozeb, maneb and mefenoxan, and the insecticide dicofol, sequential application could be a solution. It is suggested that the incompatible fungicides may be applied before or after the application of the biocontrol fungus, without adversely affecting disease development on *Amaranthus* species, as it has been done in the case of the bioherbicide Collego (Smith, 1991).

In one of the experiments, some of the adjuvants tested increased spore germination in comparison with the control [polyoxyethylene sorbitan monolaurate, acrylamide potassium acrylate copolymer, paraffinic petroleum oils/light mineral oil, 2-propenamide copolymer (2) and the algal polysaccharide]. However, this result was not confirmed in the repeat experiment and might be related to experimental variation such as overlaying of spores and following inhibitory activity. The adjuvants that completely inhibited or reduced spore germination were either an alkylate (alkylaryl polyethoxylate) or ether based (polyalkyleneoxide-modified heptamethyltrisiloxane and nonoxynol). Pesticides that contain or require addition of these adjuvants should not be tank-mixed with *P. amaranthicola*. However, in the study of Vincent and Charudattan (1999), the polyalkyleneoxide-modified heptamethyltrisiloxane was compatible even at 0.05% and 1% under greenhouse and field conditions, respectively, with mycelium of *Myrothecium roridum* Tode ex Fr. and *Cercospora rodmanii* Conway (= *C. piaropi* Tharp emend. Conway emend. Tessman *et al.*), potential biocontrol agents for water hyacinth. Furthermore, the polyalkyleneoxide-modified heptamethyltrisiloxane improved the frequency of penetration on *Pteridium aquilinum* (L.) Kühn by *Ascochyta pteridis* Bres., but disease development was delayed. In this case, Womack and Burge (1993) observed phytotoxicity of the surfactant to the guard cells, which may have induced a fungicidal microhabitat in the manner of a hypersensitive response. Zidack and colleagues (1992) observed a promotion of bacterial infection of leaves by the polyalkyleneoxide-modified heptamethyltrisiloxane due to its ability to penetrate stomatal openings. Most of the adjuvants we tested were non-ionic, except the blend of anionic and non-ionic components. However, it is assumed that the anionic component has no influence on spore germination. All other adjuvants can still be considered as compatible also if germination of conidia was reduced by 20% in comparison with the control. Such a reduction can be compensated for by using a slightly higher inoculum level because high levels of disease incidence and subsequent disease severity by *P. amaranthicola* results from about 100 000 spores mL^{-1}.

It has to be mentioned that combinations of certain pesticides and adjuvants can have effects on spore germination that are unlike those resulting from pesticides alone or adjuvant alone (Smith & Hallett, 2003). However, we did not test the effects of different combinations of pesticides and adjuvants on the fungus. Therefore, additional studies should be carried out with selected combinations of pesticides and adjuvants used in crop production.

These data provide a guide for the compatibility/incompatibility of *P. amaranthicola* with herbicides, fungicides and insecticides. This information can be used to develop recommendations for applications of the bioherbicide and chemical pesticides. We believe that the effect pesticides have upon spore germination is more important than on mycelial growth because spores are the source of disease initiation and spread. It is possible that the test chemical is not inhibitory to conidial germination and the germinating mycelium may metabolize and convert the chemical to a fungi-toxic fraction. This will stop further growth. It is obvious from this study that the effect of chemicals on spore germination do not fully correspond with those on already established mycelium on agar plates. Although these results of the *in vitro* effects of the chemicals may not necessarily correlate with their effects under field conditions, they provide a useful guide for selection of appropriate systems for integration of *P. amaranthicola* with chemical pesticides. *In vitro* tests serve as starting points to test for compatibility of different chemicals with mycoherbicide agents. Generally, *in vitro* tests provide fairly reliable estimates of compatibility that can be further tested and validated with *in planta* studies.

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